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Characterization of red-spotted grouper nervous necrosis virus isolated from ovarian fluids of asymptomatic wild Asian seabass, *Lates calcarifer*

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ABSTRACT

Viral nervous necrosis (VNN) caused by nervous necrosis virus is a serious disease affecting a wide range of marine, brackishwater and freshwater fishes. The disease causes acute mortalities in larval and early juvenile stages while adult fishes are asymptomatic and remain as carriers of the virus. In the present study, nervous necrosis virus was isolated from ovarian fluids of asymptomatic wild-collected Asian seabass, *Lates calcarifer* in SSN-1 cell line. The complete genome of the virus was amplified by PCR from plaque purified virus and sequenced. Bioinformatic analysis of the sequence revealed that the isolate belonged to red-spotted grouper nervous necrosis virus. The virus purified from infected SSN-1 cell culture supernatants by cesium chloride gradient ultracentrifugation resulted in two bands in the gradient. Both the bands on SDS-PAGE analysis and western blot revealed a single 40 kDa capsid protein. The virus replication was optimum at 26–28 °C *in vitro* and the virus can be completely inactivated using 3 mM binary ethylenimine in 32 h at 25 °C. The isolated virus was infectious and caused 100% mortality in Asian seabass larvae. The inactivated virus when injected to Asian seabass fingerlings produced neutralizing antibody titre ranging from 1:320 to 1: 1280. The isolate is a potential candidate for production of an inactivated vaccine against VNN.

1. Introduction

Viral nervous necrosis (VNN) also called as viral encephalopathy and retinopathy (VER) is an acute viral disease affecting more than 120 species of marine, brackishwater and freshwater fishes belonging to 30 families from 11 different orders (Costa and Thompson, 2016). The disease was first reported in hatchery-reared Japanese parrotfish, *Oplegnathus fasciatus*, larvae and juveniles, in Japan (Yoshikoshi and Inoue, 1990) and larvae of barramundi, *Lates calcarifer* in Australia (Glazerbrook et al., 1990). The disease causes acute mortality in fry and juveniles while adult fish are relatively less susceptible. The commonly observed clinical symptoms of VNN are abnormal swimming, which includes whirling, spiral swimming, darting and loss of appetite. Skin pigmentation abnormalities and hyperinflation of swim bladder can also be observed in diseased animals (Bandín and Souto, 2020). VNN is caused by nervous necrosis virus (NNV), a betanodavirus belong to the family *Nodaviridae*. It is a non-enveloped virus measuring about 25 nm in diameter and the genome consists of two segments of single-stranded positive-sense RNA both lacking poly(A) tail (Mori et al., 1992). The RNA1 is approximately 3081 bases long coding for a 110 kDa RNA dependant RNA polymerase (RdRp) or protein A which directs spontaneous replication of the viral genome during infection (Delsert et al., 1997; Nagai and Nishizawa, 1999). The RNA2 is 1410 bases long containing a single coding region of 1023 bases for a 340 amino acid coat protein of 42 kDa in which the genomic RNA molecules are packaged (Mori et al., 1992; Nishizawa et al., 1995). The capsid protein is glycosylated and hence the molecular weight observed in SDS-PAGE gels is 5 kDa more than the deduced molecular weight of 37 kDa (Chi et al., 2001). RNA3, a sub-genomic transcript of RNA1, of size 371–378 bases codes for two non-structural proteins B1 and B2 having roles as anti-necrotic death factor and in supressing cellular RNA interference, respectively (Chen et al., 2009; Fenner et al., 2006; Iwamoto et al., 2005; Sommerset and Nerland, 2004).

The fish nodavirus are classified into four genotypes based on the phylogenetic analysis of the T4 variable region comprising of 427 bases of RNA2 sequence of 25 isolates viz., tiger puffer NNV (TPNNV), striped

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